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Original Scientific Paper

CORRELATION OF EXPRESSION OF TGF- β AND MMP2 BETWEEN PROSTATIC ADENOCARCINOMA AND ADJACENT UNAFFECTED PARENCHYMA

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SUMMARY: In prostate adenocarcinoma, both tumorous stroma and epithelium have important role in tumor progression. Transforming growth factor beta (TGF- β) is a promotor in advanced stages of prostate cancer. Matrix Metalloproteinase 2 (MMP2), the endopeptidase that degrades extracellular matrix is considered to be overexpressed in prostatic carcinoma related to its growth and aggressiveness. Therefore, the aim was to analyze the expression of proteins TGF- β and MMP2 between both epithelium and stroma of prostatic adenocarcinoma and adjacent unaffected parenchyma. The intensity of TGF- β and MMP2 expression in epithelium, tumorous stroma and adjacent unaffected parenchyma was analyzed in 62 specimens of prostatic adenocarcinoma by microarray-based immunohistochemistry. TGF- β was more expressed in tumorous than in prostate stroma (p =0.000), while no statistical significance in case of MMP2 (p = 0.097) was found. MMP2 was more expressed in tumorous than in prostate epithelium (p =0.000), while no statistical significance in case of TGF- β (p = 0.096) was observed. The study results indicate that both tumorous stroma and epithelium have a role in tumor progression and support potential role of TGF- β and MMP2 in prostatic adenocarcinoma progression.

Key words: *TGF- β, MMP2, prostatic adenocarcinoma, unaffected parenchyma*

Introduction

According to current WHO classification of tumors of the urinary system and male genital organs, prostatic adenocarcinoma is the second most common malignancy in males (1). It is also the fifth cause of lethal outcome in male population worldwide (1). Many factors including Transforming growth factor beta (TGF- β) and Matrix Metalloproteinase 2 (MMP2) are considered to be deregulated and included in prostate carcinoma progression. TGF- β is a peptide responsible for cell proliferation, differentiation, migration and apoptosis regulation (2,3). In normal prostatic epithelium and in early stages of prostatic cancer, its role includes the induction of apoptosis and inhibition of cell proliferation, while in advanced stages of cancer, it becomes its promotor (2). MMP2 is a zinc- dependent proteinase

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important for the degradation of extracellular matrix (proteoglycans, glycoproteins, fibrillar and nonfibrillar collagen, denatured collagen) and is considered to be overexpressed in prostatic adenocarcinoma related to its growth, aggressiveness, disease stage and Gleason score (4,5). Its overexpression has also been noted in gastric, breast, ovarian, urothelial, pancreatic, lung and colon neoplasms where it was related to poor prognosis and shorter survival (4,5). The aim of this study was to investigate the expression of proteins TGF- β and MMP2 in both epithelium and stroma of prostatic adenocarcinoma and adjacent unaffected parenchyma.

Patients and methods

The samples of 62 patients with prostate adenocarcinoma who underwent radical prostatectomy were collected and archived in the Ljudevit Jurak Clinical Department of Pathology and Cytology in the Sestre milosrdnice University Hospital Center, Zagreb, Croatia, during the year 2016- 2018. The diagnosis of prostatic adenocarcinoma was established through needle core biopsy and confirmed after radical prostatectomy. Patient median age was 65.2 (52-75). Gleason score 6 was present in 11 (17,7%) patients, score 7 (3+4) in 32 (50%), score 7 (4+3) in 5 (8,1%) patients, 12 (19,4%) with score 8, score 9 (4+5) was observed in 2 (3,2%) patients. The samples were subsequently analyzed by microarray-based immunohistochemistry. The immunostaining pattern in all analyzed markers was cytoplasmic, scored from 0-3 and expressed as score 0 (up to 25% of positive carcinoma or stromal cells), score 1 (›25%-50% of positive carcinoma epithelial/stromal cells), score 2 (›50%-75% of positive carcinoma epithelial/stromal cells) and score 3 (more than 75% of positive carcinoma epithelial/stromal cells). The same scores were applied in the analysis of these markers' expression in epithelial and stromal cells of unaffected parenchyma. A statistical analysis was performed by using Wilcoxon Signed Ranks Test. The analysis of Gleason score correlation with TGF- β and MMP2 expression in malignant epithelium and stroma was done by using Spearman test. The results were considered statistically significant when $p<0.05$.

Results

Cytoplasmic TGF- β staining was observed in 57 samples of tumorous epithelium, in 52 samples of

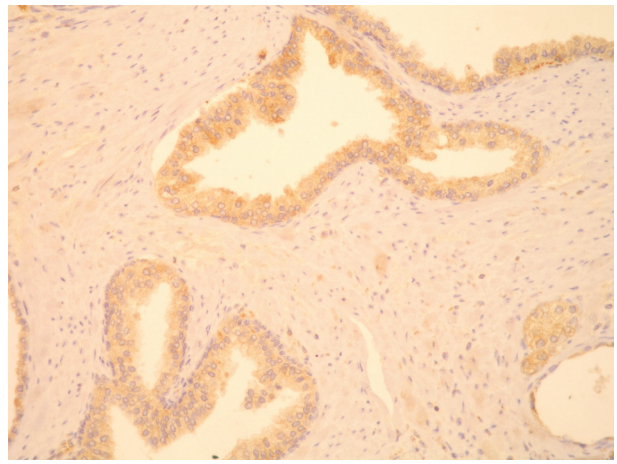

Figure 1. TGF-β in non- tumorous prostate epithelium and stroma (TGF-β x 200).

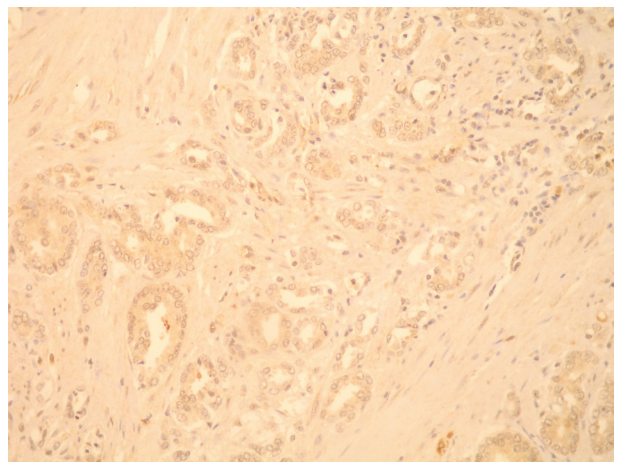

Figure 2. TGF- β in tumorous prostate epithelium and stroma (TGF-β x 200).

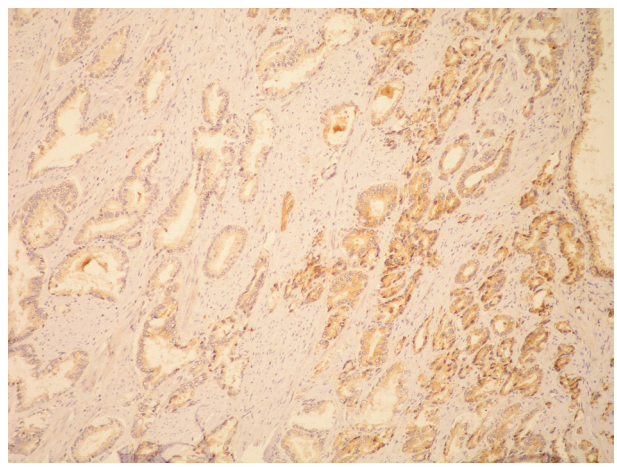

Figure 3. MMP2 in tumorous vs. non- tumorous epithelium (MMP2 x 100).

unaffected epithelium of adjacent prostate tissue, 50 samples of tumorous stroma and 36 samples of non-tumorous stroma (Figures 1 and 2). In case of cytoplasmic MMP2 expression, the tumorous epithelium demonstrated positive staining in 61 samples, non-tumorous epithelium in 53 samples, in 14 samples of tumorous stroma and 7 samples of non- tumorous stroma (Figure 3). The obtained results indicated that TGF- β was more expressed in tumorous than in non-tumorous stroma (p =0.000), while there was no statistical significance found in case of MMP2 (p = 0.097) (Table 1). MMP2 was more expressed in tumorous than in non- tumorous epithelium (p =0.000), yet no statistical significance in case of TGF- β (p = 0.096) was determined (Table 2). There was no statistical significance between Gleason score and the expression of TGF- β found in tumorous epithelium (p=0.776) and stroma (p=0.305). Furthermore, no statistically significant correlation between MMP 2 expression in tumorous epithelium (p=0.281), tumorous stroma (p=0.993) and Gleason score was established (Table 3).

Table 1. Correlation of expression of MMP2 and TGF-β between tumorous and non- tumorous stroma

	non- tumorous stroma – tumorous stroma	
	MMP2	TGF-β
Z	-1.661	-3.734
P value	.097	.000

Wilcoxon Signed Ranks Test

Table 2. Correlation of expression of MMP2 and TGF-β between tumorous and non- tumorous epithelium

	non- tumorous epithelium – tumorous epithelium	
	MMP2	TGF-β
Z	-4.129	-1.664
P value	.000	.096

Wilcoxon Signed Ranks Test

Discussion

Nowadays, the detection of prostate cancer in patients has become more successful, mostly due to the determination of PSA levels in combination with mpMR and needle core biopsy, however, the prognosis of metastatic prostate cancer is still unsatisfactory creating thus the need to investigate factors included in invasion and metastasis of this carcinoma (6). It is also well known that prostate adenocarcinoma is composed not only of malignant epithelial cells, but also of supportive stroma containing fibroblasts, myofibroblasts, immune and endothelial cells. The interactions between epithelial and stromal components form a microenvironment are necessary for tumor differentiation and proliferation (7). Carcinoma cells have impact on fibroblasts by turning them to reactive myofibroblasts which secrete growth factors, proteolytic enzymes and extracellular matrix components, promoting thus carcinoma progression (7). Cancer- associated fibroblasts being the main component of prostate tumor stroma interact with tumor epithelial cells triggered by paracrine factors and promote carcinogenesis and cancer progression (8). One of the important substances secreted by tumor is cytokine TGF- β (8). In normal prostate, TGF- β affects the differentiation of the cells and inhibits epithelial cell proliferation through apop-

Table 3. Correlation of Gleason score and the expression of TGF-β and MMP2 in tumorous epithelium and stroma

		TGF-β in tumorous epithelium	TGF-β in tumorous stroma	MMP2 in tumorous epithelium	MMP2 in tumorous stroma
Gleason score	Correlation Coefficient	-.037	-.132	.139	.001
	N	62	62	62	62
	P value	.776	.305	.281	.993

Spearman correlation test

tosis induction (9). This factor has complex functions and an important role in prostate carcinoma since it is involved in its development and progression (10). In prostate cancer, the levels of TGF- β are increased and accompanied by a loss of TGF- β receptor resulting in the lack of apoptosis and more proliferative activity, and consequently in poorer prognosis (9). In healthy prostate, the balance is created between TGF- β and androgen signaling pathways, while in prostate carcinoma, the androgens in epithelial cells stimulate TGF-β production, but decrease the expression of TGF- β-RII receptor creating a resistance to apoptosis (9). In prostate cancer, the levels of TGF- β in stroma are increased, which contributes to extracellular matrix remodeling, transformation of fibroblasts to myofibroblasts and epithelial- mesenchymal transition facilitating the progression and spread of tumor (9). The results of our research support these findings, demonstrating significantly higher expression of TGF- β in tumorous stroma than in non- tumorous stroma. TGF- β signaling has an important role not only in prostate cancer, but also in colorectal, breast, liver, pancreatic cancer since its dysregulation contributes to oncogenesis through a lower degree of apoptosis, increased cell proliferation and induction of epithelial-to-mesenchymal transition (2,10). During tumor progression, carcinomatous cells develop resistance to TGF- β growth restriction arising from mutations and inactivation of TGF- β signaling pathway. (10) TGF- β also expresses immunological function in tumor biology by inhibiting proliferation of T cells and creating more suitable environment for the spread of tumor (9). According to literature, the initial suppressive role of TGF- β and its later promotor role in prostate cancer is achieved by its interactions with microRNAs (10). The change of microRNAs expression levels between primary tumor and metastasis is believed to be the possible reason for dual behavior of TGF- β in carcinoma, and clinical studies involving therapeutic microRNAs are currently ongoing (10). According to all its functions, it is believed that blocking TGF- β would lead to the inhibition of tumor progression and metastasis (10). Matrix metalloproteinases are generated in prostate adenocarcinoma by both epithelial and stromal cells of the tumor giving rise to the role of enzymes completing matrix degradation, but also controlling tumor growth, facilitating invasion through the loss of cell adhesion, promoting angiogenesis through their influence on signaling pathways (7,11,12). Currently, MMP's are recognized by number since they affect multiple substrates, making thus the previous classification based on the substrate specificity inadequate (13). The regulation of MMP's activities is performed on both transcriptional and post- transcriptional level and influenced by oncogenes, growth factors and cytokines (11,14). MMP's engage also in the interactions among each other and other proteolytic enzymes in order to gain activation and proteolytic activity, where MMP2 appears to be one of the most important enzymes (14). MMP's also influence the generation and respond to signaling molecules at the surface of the cells (13). MMP2 is an important factor enabling tumor cells migration through its principal role in degradation of collagen I, IV, V, VII, X, XI, fibronectin and laminin (6,15). It was demonstrated as overexpressed in different neoplasms where it contributes to increased tumor aggressiveness through its proteinase activity and activation of other proteinases (4,16). In prostate cancer, its overexpression by malignant prostatic epithelium is considered to contribute to increased tumor invasiveness, migration, metastasis, higher tumor stage, higher Gleason score and is related to shorter disease- free survival, but it is also involved in prostate cancer development (4,5,15). It is also believed that MMP2 rs243865 polymorphisms are related to higher risk of prostate adenocarcinoma and metastasis (17). MMP2 and TGF- β are closely related since MMP2 stimulates EMT through the activation of TGF- β by solubilizing TGF-β of extracellular matrix (13). The results of our study were in concordance with the findings of MMP2 overexpression in tumorous epithelium, however, there was no significant correlation between Gleason score and its expression demonstrated, but we believe that it would be recommendable to include a larger number of samples. To our knowledge, a possible effect of MMP inhibitors could be useful if applied in early stages of cancer in combination with other therapeutic modalities (13). Since metastatic spread of prostate carcinoma is the key point that leads to lethal outcome (15), we are considering the research and therapeutic interventions including factors such as MMP2 and TGF- β necessary.

In conclusion, our study results demonstrate that both tumorous stroma and epithelium have a role in tumor progression and support potential role of TGF-β and MMP2 in prostatic cancer progression. Howev-

er, we believe that further research of possible therapeutic approach to these markers is needed.

Authors declare no conflict of interest.

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Sažetak

KORELACIJA EKSPRESIJE TGF-β I MMP2 IZMEĐU ADENOKARCINOMA PROSTATE I OKOLNOG NEZAHVAĆENOG PARENHIMA

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U progresiji karcinoma prostate tumorska stroma i epitel imaju važnu ulogu. Beta transformirajući faktor rasta (TGF-β) djeluje kao promotor u uznapredovalom raku prostate, a matriks metaloproteinaza 2 (MMP2) kao endopeptidaza koja razgrađuje izvanstanični matriks te je njezina aktivnost pojačano izražena te povezana s rastom i agresivnošću ovog tumora. Stoga je cilj ovog istraživanja bio analizirati izraženost TGF- β u MMP2 između epitela i strome karcinoma prostate te okolnog tumorom nezahvaćenog parenhima prostate. Intenzitet izraženosti TGF- β i MMP2 u tumorskom epitelu i stromi te okolnom nezahvaćenom parenhimu je analiziran u 62 pacijenta s adenokarcinomom prostate metodom imunohistokemijske analize na uzorcima microarray-a. TGF- β je bio izraženiji u tumorskoj nego u ne- tumorskoj stromi (p =0.000), dok u slučaju izraženosti MMP2 nije pokazana statistička značajnost (p = 0.097). MMP2 je bila izraženija u tumorskom epitelu u odnosu na ne- tumorski epitel (p =0.000), dok statistička značajnost u slučaju izraženosti TGF- β nije pokazana (p = 0.096). Rezultati ovog istraživanja pokazuju da i tumorski epitel i stroma imaju ulogu u progresiji tumora i ukazuju na potencijalnu ulogu TGF- β i MMP2 u progresiji adenokarcinoma prostate.

Ključne riječi: *TGF- β, MMP2, adenokarcinom prostate, ne- tumorski parenhim*